Rapid Adaptation to Mammalian Sociality via Sexually Selected Traits

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Abstract

Background

Laboratory studies show that the sexual selection via mate choice and intrasexual competition can profoundly affect the development and fitness of offspring. Less is known, however, about the total effects of sexual selection on offspring in normal social conditions. For many animals, opportunity for mating success is determined by complex social interactions, such as dominance hierarchies. Social selection is an extended view of sexual selection that incorporates competition during sexual and nonsexual interactions, and predicts complex evolutionary dynamics. Whether social selection improves or constrains offspring fitness is controversial.

Results

To characterize the consequences of social selection, we introduced wild-derived mice to seminatural competition for three consecutive generations (promiscuous lineage). In parallel, we bred a control lineage in cages using random mate assignment (monogamous lineage). A direct competition experiment using second-generation animals revealed that promiscuous line males had greater reproductive success than monogamous line males (particularly during extrapair matings), in spite of higher mortality and equivalent success in social dominance and sperm competition. There were no major female fitness effects, though promiscuous line females had fewer litters than monogamous line females. We confirmed a behavioral sexual attraction mechanism by showing that, when given a choice, females had both odor and mating preferences for promiscuous line over monogamous line males.

Conclusions

Our study demonstrates novel evidence for the strength of sexual selection under normal social conditions, and shows rapid male adaptation to sociality driven largely by sexual trait expression, with tradeoffs in survivorship and female fecundity. The speed at

47 which these phenotypes emerged suggests the possibility of transgenerational
48 inheritance. Mouse population densities fluctuate dramatically in nature, and we propose
49 that sexually selected phenotypes arise dynamically during periods of social competition.
50

Background

The relative importance of the indirect effects of sexual selection on offspring fitness is the subject of ongoing debate [1-4]. By eliminating other forms of reproductive competition, animal laboratory experiments have revealed that mate choice can strongly affect offspring fitness. For example, in a 10 minute assay, female mice mated to males they preferred to associate with in a two-way mate choice apparatus had offspring with greater viability than females mated to males they did not prefer to associate with [5]; similar results were found in a male association preference assay [6]. Intrasexual competition can also have independent effects on offspring fitness, and individuals with high competitive ability often have offspring with higher fitness than their non-dominant counterparts [7]. These studies show that sexual behaviors affect offspring fitness under certain conditions. Less understood, however, is whether these results are emblematic of the cumulative effects of sexual selection in normal social conditions [3].

For social animals, sexual selection takes place within a complex social network. The opportunity for sexual selection is largely determined by the structure of the social environment because relatively few socially dominant individuals are able to monopolize reproduction [8, 9]. Thus, social selection, an extended view of sexual selection, refers to differential mating success due to any form of social competition, including competition over social rank, food, resources, space, parental care or kinship [10, 11]. Indirect fitness effects of social selection could improve or constrain offspring fitness in competitive populations. For instance, the social environment can impose constraints on mating decisions such that individuals are forced to mate with partners they would otherwise not prefer, resulting in decrements to offspring viability [5, 12]. Alternatively, competition within the social environment could improve the opportunity for sexual selection by providing maximal information regarding the potential fitness of offspring [13, 14].

Empirical measures of selection in socially competitive environments are mixed. In the fruit fly *Drosophila melanogaster*, there is abundant evidence that larvae conceived in socially competitive environments have a fitness disadvantage [15-17], though the opposite effect has also been observed [13]. Studies on flour beetles [18] and amphipods [19] suggest that mating in socially competitive arenas produces sex-specific indirect effects, where fitness is enhanced in sons and depleted in daughters. Missing from these experiments, however, is a naturalistic social context [20]. In the wild, for instance, *D. melanogaster* males compete for a limited number of positions on the feeding site, which is where female choice is normally exercised [21, 22]. More studies are needed to understand how parameters of offspring fitness affect the dynamics of social and sexual selection.

There are three primary theories for the evolution of sexually selected traits [23]. First, the good genes model predicts that viability, usually in males, is signaled through the expression of condition-dependent traits and preference evolves because choosy females produce offspring with higher viability. Zahavi's handicap principle [24] is a kind of good genes model, and suggests that the expression of secondary sexual traits comes at the cost of survivorship, so that only highly fit individuals are able to afford to display "cheat proof" handicap signals. Second, the sexy sons model predicts that coevolution of female choice and male traits can become linked such that males who produce attractive sons, but otherwise make no contribution to offspring viability, will be preferred by females. Third, the chase-away model suggests that male display traits can originate via exploitation of preexisting sensory bias in females, who are induced to mate in a suboptimal manner. Females can then evolve resistance by no longer preferring the trait, and males are in turn selected to evolve an even more extreme display trait. This latter model is a specific case of sexual conflict, which predicts that, due to the divergent

reproductive interests of the sexes, promiscuity can lead to selection on traits that increase fitness in one sex at the expense of fitness in the opposite sex [25].

Social and sexual selection pressures can also influence offspring fitness through mechanisms that cannot be attributed to inherited alleles (i.e. transgenerational effects) [4, 26]. Parents, particularly mothers, can alter investment in offspring development in response to social cues or environmental stressors such as resource availability, and in turn affect offspring social competitiveness [27, 28]. Although parental effects can adaptively prime offspring for the current environment, conflicts between optimal strategies of parents and offspring (e.g. in resource allocation) can also incur costs on offspring [26]. How inheritance mechanisms collectively respond to social selection is not well understood, again highlighting the need for empirical measures of indirect effects of social and sexual selection.

Wild house mouse (*Mus musculus*) populations range from few to hundreds of individuals per acre, and transitions between these density extremes frequently occur within a few reproductive cycles [29, 30]. Low-density populations lack social structure, while high-density commensal populations are organized around discrete territories consisting of a socially dominant male and several dominant females [31, 32]. Social and sexual selection should be more intense in these high density environments because mate choice is largely driven by females, who typically mate with the locally dominant male and/or dominant males of neighboring territories [33].

We report here a breeding experiment to assess the indirect effects of social and sexual selection in mice. Motivated by the observation that mate preference alone confers substantial benefits to offspring [6, 34], we aimed to identify whether the effects of competition within a social environment also improved offspring fitness, and if so, to what degree and through which components of fitness. We predicted that, although mating decisions in high-density populations might be constrained by social dominance

hierarchies, females would nevertheless select mates that increased the fitness of their offspring. We bred a “promiscuous” line in socially competitive enclosures where mice compete for mates, nesting sites, and social dominance, and a “monogamous” line where social selection is eliminated by caged, random mate assignment [35].

We bred monogamous and promiscuous mice within their designated social treatment for three consecutive generations. During breeding, promiscuous line females were transferred to cages for parturition to minimize environmental effects on offspring fitness. In the first test, we directly competed second-generation monogamous line and promiscuous line mice in enclosures to measure lifetime fitness and found that the promiscuous line had a fitness advantage over the monogamous line through increased male reproductive success, which was almost exclusively due to an advantage during extrapair matings. To identify the role of female preference in this fitness effect, we then used third-generation mice in a laboratory mate choice assay and found that females had both odor and mating preferences for promiscuous line males over monogamous line males. These results show that social selection in mice has rapid effects on male offspring and favors the expression of secondary sexual traits.

Methods

Breeding design and mice

Mice used in this experiment were subjected to independent iterations of either enforced monogamy in cages or social selection in a seminatural environment where breeding is promiscuous. Components of natural selection such as fecundity and viability were equalized by taking a roughly equal number of offspring from an equal number of litters to found the subsequent generation; similarly, both treatments were equally exposed to potential pathogens that may have been present in the animal facilities.

The breeding design for this experiment is illustrated in **Figure 1**. Initially, 50 P_0 males and 50 females (age-matched and avoiding inbreeding) were first bred monogamously and allowed to produce a single litter, which established the monogamous lineage. The same 100 adults were then randomly assigned to three independent seminatural population enclosures. Pups from first litters conceived in the enclosures established the promiscuous lineage. (There were no differences between the size of first and second litters produced by P_0 females, as measured by the number of pups that survived to weaning age (means ± s.e.m.: first = 6.81± 0.376; second = 7.17 ± 0.381)). Within each breeding treatment, random subsets of the resulting offspring were weaned at three weeks of age and used as the parental population for the subsequent generation, for a total of three generations. Both lineages were maintained at a potential effective population size (N_e) of 100, but since some individuals do not breed due to social competition, the realized N_e of the promiscuous line was below 100.

Throughout, enclosure populations consisted of, on average, 10 males and 20 females to reflect the female biased adult sex ratio commonly found in commensal populations [32]. To minimize differential effects of experience and environment on offspring from the promiscuous and monogamous lines, pregnant females from the promiscuous line were transferred from enclosures to cages for parturition. Because nongenetic effects may be a crucial mechanism for rapid adaptation to changes in the social environment, this design does provide opportunity for transgenerational epigenetic inheritance [36] and maternal effects [37] by directly exposing parents to the two different social conditions. Experimental comparisons between treatments (i.e. direct competition and mate choice experiments, below) were therefore performed on cage-born offspring from both lines; in other words, the promiscuous offspring had never experienced seminatural conditions, only their ancestors had.

Seminatural enclosures

Our seminatural enclosures mimic the social ecology of *Mus domesticus* in nature; the incidence of multiple paternity [38], population density [30], and sex ratio [31] are all consistent with measurements from natural populations. Each enclosure (ca. 22.2 m^2) was divided into six subsections by climbable hardware cloth (45.72 cm) to create territorial boundaries. Four subsections were designated as "optimal territories" and had defendable nest boxes made of covered, opaque plastic bins (75.7 liters) with 5.08 cm diameter entryways and containing nesting materials and food. The remaining two subsections were designated as "suboptimal territories" that had light-exposed nest boxes (made of 61 cm x 20 cm planter boxes fitted with wire lids and 5.08 cm entryways) and adjacent open-access food containers. Together, optimal and suboptimal territorial boundaries created environmental complexity in which mice established social dominance hierarchies. Water was provided ad libitum in one-gallon poultry waterers. This type of seminatural enclosure has been used in a variety of experiments [39-42] and elicits normal behavior of wild mice.

To prevent incidental breeding before the establishment of male social territories (the primary social unit in commensal mice), placeholder females were used at the onset of each population for one week before promiscuous line breeding females replaced them in the enclosures.

Direct competition (F_2)

To determine whether animals from the promiscuous line gained a fitness advantage following two generations of sexual selection, we competed F_2 cage-born promiscuous and monogamous (n = 300) mice in 10 independent enclosures for 35 weeks. Each competition enclosure consisted of 10 males and 20 females; equal portions came from the monogamous and promiscuous lines. The influence of

204 inbreeding was controlled by limiting the presence of relatives within an enclosure to a
205 few pairs of female cousins, which were always equally balanced between treatments.
206 *Reproductive success.* Treatment-level reproductive success was determined by
207 periodic (approximately every 35 days) “offspring sweeps” (n = 5) in which pups born
208 during the previous cycle were sacrificed and tissue samples were taken for genetic
209 analysis. Reproductive success was determined by analysis of genetic polymorphisms
210 segregating in this population as described previously [41]. Briefly, in each competition
211 enclosure male and female founders of each treatment were grouped by the presence of
212 a shared microsatellite allele at a polymorphic locus on the Y chromosome and a length
213 polymorphism in mitochondrial genome, respectively. Accordingly, treatment-level
214 reproductive success for male founders was determined by genotyping male offspring,
215 and female reproductive success was determined by genotyping all offspring.
216 Litters were classified by their precise location, estimated age of the pups, and
217 their mitochondrial genotype. “Regular” litters had a single mitochondrial genotype, were
218 found in one location and showed a uniform age. “Mixed” litters had two mitochondrial
219 genotypes, were found in multiple locations, had different age classes, or any
220 combination thereof. “Communal” litters had more than 12 offspring of the same age.
221 Litters were also classified by the presence of one or two Y chromosome markers, and
222 identified a subset of litters sired by multiple males. These data were used to determine
223 treatment effects on reproductive success, treatment × sex interactions, and the role of
224 social dominance in reproductive success.
225 *Parentage analysis.* To determine individual-level reproductive success, we
226 used paternity analysis by scoring parents and offspring from two populations using 10
227 unlinked autosomal microsatellite loci, as described previously [43]. Parentage was
228 estimated using Cervus likelihood analysis software [44]. All parent offspring trios were
229 found to have greater than 80% confidence. Identification of “mixed” (i.e., multiple

paternity) litters was used to determine the effect of breeding treatment on reproductive success during postcopulatory sexual selection (sperm competition and cryptic female choice). We also analyzed the effects of breeding treatment on the size and number of "pure" (i.e., sired by a single male) litters. Paternity data were used in conjunction with social dominance data to determine whether breeding treatment affected within- and extra-territorial mating success.

Social dominance. To determine social dominance and territoriality, founders were marked with unique passive integrated transponder (PIT) tags surgically implanted subdermally between the scapulas. Transceivers and readers (BioMark, Boise, ID) were placed at each of the optimal and suboptimal feeders in two enclosures at a time, and data streamed to a computer with data-logging software (Minimon, Culver City, CA). Transceivers were regularly rotated through the 10 enclosures throughout the competition experiment. Male social dominance was assigned when a male had >90% of the PIT-tag reads at a single reader. At "undefended" locations no single male had 90% of the reads. Females move between territories more than males, and though they do not compete for exclusive representation at a single location, they do compete for positions at optimal territories as indicated by female-female agonistic interactions and higher reproduction in optimal territories (below). Thus, female social dominance was assigned when >50% of a given female's reads were at a single location.

PIT-tag data were used to determine treatment effects on social dominance ability in three ways. First, we compared the frequency of socially dominant males and females between treatments. Second, we used treatment-level (Y chromosome and mitochondrial genotyping) reproductive success measures to identify treatment effects on reproduction within defended and undefended territories. Third, because we found that promiscuous line males had an advantage in reproductive success over monogamous line males, we subsequently used parentage analysis to investigate the

number of offspring born within the territory of their socially dominant father ("within-territory"), outside the territory of their socially dominant father ("extra-territory"), or in territories undefended by a dominant male ("undefended").

Survivorship. Survivorship of founders (n = 300) was determined by periodic checks (approximately every 10 days) in each enclosure. Dead founders were identified by their PIT-tag.

Major Histocompatibility Complex. The major histocompatibility complex (MHC) is a substantial mediator of vertebrate fitness effects [45]. The mouse strain used is this experiment carries an outbred, wild-derived genetic background on which five known MHC haplotypes from laboratory strains have been introgressed through selective breeding. When assigning monogamous line and promiscuous line mice to competition enclosures, we used this genetic information to balance MHC allelic diversity between each treatment. As a result, MHC haplotype frequencies were equivalent between treatments at the onset of competition. By controlling for the opportunity of MHC-mediated selection, this strategy allowed us to better focus on the effects of social selection for two reasons. First, MHC-mediated mate choice (either due to heterozygote advantage, rare-allele advantage, or inbreeding avoidance) is well documented to have a profound fitness effects in both socially competitive and noncompetitive environments [46]. Second, MHC-mediated selection resulting from pathogen pressures could have introduced differential disease susceptibility profiles between the monogamous and promiscuous lines during the three generations of breeding [47]. Thus, by balancing MHC genotypes between treatments, we ensured that MHC-mediated selection would not obscure the effects of social selection.

Mate choice (F_3)

We used a three-cage mate choice arena and the Timescience (Salt Lake City, UT) recording system to determine if the promiscuous line male fitness advantage during direct competition was driven by female odor and mating preferences. The mate-choice arena consisted of two “male” cages connected by PVC tunnels to a single “female” cage (dimensions: 46 x 30 x 15 cm). Infrared, black and white cameras were mounted above each of three arenas. Each odor/mate preference trial consisted of two males and one female. Females were from both the promiscuous line (n = 6) and the monogamous line (n = 5). Males were given plastic collars, made of two small connected zip-ties whose protruding ends prevented them from leaving their designated cage. Prior to the experiment, males and females were housed individually in maintenance cages for one week and males were habituated to their collar for one day. Promiscuous line and monogamous line males were placed in alternating sides of the arena between trials to prevent bias. Males were age and weight matched, were unrelated to the female and to each other, and spurious effects of MHC-mediated mate choice were eliminated by ensuring that MHC haplotypes were equally represented in promiscuous line and monogamous line males.

This assay proceeded in three phases using F_3 cage-born males from the promiscuous and monogamous lines as subjects. First, to assess male scent-marking behavior, we introduced one collared male from each treatment to each of two “male” cages of the arena. Plastic collars prevented the males from leaving their designated cages, which were connected to the “female” cage by PVC tunnels. The males were allowed to scent mark the filter paper substrate of their cages for 30 minutes in the presence of a stimulus (soiled bedding from the test female’s sibling cage presented in an aerated canister). The number of, and area covered by, scent marks was quantified

using a Typhoon scanner (GE Healthcare Life Sciences, Piscataway, NJ) using the 532nm ROX filter.

Second, to determine odor preferences, females were allowed to investigate the scent marks with the males removed from the arena (females did not wear collars). Time spent investigating scent marks was quantified. Third, to determine mating preferences, collared males were reintroduced to their cages in the arena; food, water and bedding were added to each cage. After four days, females were removed from the arena and the paternity of offspring from pregnant females was determined by genetic analysis as described. Due to a slow recording rate (frame per second) over the four-day experiment, intromissions could not be discriminated from ejaculations; thus, mating preference was determined by the proportion of offspring sired by either male. All data were collected by participants blind to the treatment of the subjects.

Weight (F_3)

To test for effects of breeding treatment (promiscuous versus monogamous) on male and female weight, we recorded the mass of offspring 10 days after birth and then at 10-day intervals until the offspring were 60 days old. An average of 10 weighted individuals per treatment at each time point is reported.

Statistics

For the direct competition experiment, the effects of breeding history on reproductive success were analyzed with general linear mixed models (GLMM) using a restricted maximum likelihood approach. Reproductive success (number of offspring, number of litters and litter size) was modeled as a dependent variable for both treatment-level and individual-level data sets. Enclosure was modeled as a random factor, and pup sweep and parental treatment as fixed factors. Because socially

dominant, promiscuous line males had higher mortality than dominant monogamous line males, we also analyzed male reproductive success with number of surviving males (per treatment) as an additional factor. The interaction between male and female reproductive success was analyzed by adding sex, treatment and their interaction to a separate model. Means from each enclosure per sweep were used in the analysis. For the analysis of social dominance we modeled the number of defended territories as a dependent variable, enclosure as a random factor, and PIT-tag reader session and breeding treatment as fixed factors. To analyze population-level and individual-level effects of male social dominance on reproductive success, the mean number of male offspring per territory type was fitted as a dependent variable, enclosure as a random variable, and paternal treatment, territory type and their interaction as fixed factors.

Survivorship was analyzed with the Cox-Mantel log-rank test. For the mate choice experiments, scent marking behavior was analyzed using general linear models (GLM). Female odor and mating preferences were determined by measuring the proportion of time spent in the promiscuous line male vs. monogamous line male scent-marked cages, and the proportion of offspring sired by the promiscuous line vs. monogamous line male, respectively. Proportions were normalized by arcsine-root transformation, and comparisons were made using paired t-tests. Weight data were analyzed using GLM with weight as the dependent variable and treatment, age, and their interaction as fixed factors. Analyses were carried out with the JMP 9.0 statistical package (SAS Institute).

Results

359 Direct competition (F_2)

360 We collected 2,738 pups from 466 litters, and used 2,655 mitochondrial
361 genotypes and 1,337 Y chromosome genotypes from 10 independent populations to
362 determine reproductive success. Promiscuous line and monogamous line males had
363 57% and 43% of the offspring, respectively. Promiscuous line and monogamous line
364 females had 48% and 52% of the offspring, respectively. Results of GLMM analysis of
365 male and female reproductive success are summarized in **Table 1**. Promiscuous line
366 males produced significantly more offspring (**Figure 2a**) and a greater number of litters,
367 but did not have larger litters. Because promiscuous line males had a higher mortality
368 rate (see below), they also had greater reproductive success per surviving male than
369 monogamous line males.

370 There were no overall differences in female reproductive success (**Figure 2b**).
371 GLMM analysis showed that females from the promiscuous line had significantly fewer
372 litters, but also had slightly (but not significantly) larger litters; total number of offspring
373 was equivalent between the two treatments (**Table 1**).

374 A separate GLMM analysis with sex, treatment and their interaction as fixed
375 factors found a significant interaction effect (**Figure 2c**), but no independent effects of
376 sex and treatment (GLMM: n = 198; treatment x sex $F = 4.98$, $P = 0.027$; all other factors
377 $P > 0.24$).

378 *Social dominance and territory defense.* There were no treatment effects on the
379 ability of males to acquire and defend territories (GLMM: n = 52; treatment $F = 0.26$, $P =$
380 0.62), nor were there differences in female social dominance (n = 52; treatment $F =$
381 0.018, $P = 0.90$).

382 The majority of offspring (477/620, 77%) were born in defended territories
383 (GLMM: $n = 52$; defended $F = 46.7$, $P = <0.0001$). GLMM analysis of offspring counts
384 with paternal treatment, territory type (defended by males from the promiscuous line or

385 monogamous line, or undefended) and their interaction as factors found all three terms
386 to be significant (treatment $F = 8.23$, $P = 0.006$; territory type $F = 3.95$, $P = 0.026$;
387 interaction $F = 23.78$, $P < 0.0001$; **Figure 3**). Ninety-five percent of the offspring born in
388 territories defended by a promiscuous line male had promiscuous line paternity; in
389 contrast, just 68% of the offspring born in territories defended by a monogamous line
390 male had monogamous line paternity. Promiscuous line males also had greater
391 representation in undefended territories, with 61% of the offspring. Post-hoc Tukey's
392 HSD tests showed no additional significant effects. Thus, promiscuous line males had
393 significantly greater fitness due to greater reproductive success in all three territory
394 types.

395 *Parentage analysis of reproductive success and social dominance.* From two
396 enclosure populations we genotyped the promiscuous line and monogamous line
397 founders (n = 60) and the offspring born during competition (n = 559, comprising 110
398 litters). Consistent with treatment-level measurements, 57% of the offspring had
399 promiscuous line paternity and 47% had promiscuous line maternity. Results from
400 GLMM analysis of parentage data are summarized in electronic supplementary material
401 **Table 2**; for brevity, only significant results are reported here. First, we analyzed the
402 effects of breeding treatment on the size of “pure” or “mixed” litters. Seventy-three
403 percent of litters were of single paternity and 27% were of mixed paternity. Mixed litters
404 were significantly larger than pure litters (Means: mixed = 6.8 pure = 4.9; GLMM: n =
405 100; $F = 10.3$, $P = 0.0019$). There were no effects of paternal breeding treatment on the
406 size of mixed or pure litters (**Figures 4a, b**), nor were there effects of maternal treatment
407 or paternal *x* maternal interactions on mixed and pure litter size (electronic
408 supplementary material **Table 2**).

409 We next analyzed individual male reproductive success (from both treatments)
410 by comparing the number of offspring born in each of three conditions: within a defended

territory and a descendent of the locally dominant male (within-territory), within a defended territory but not a descendent of the locally dominant male (extra-territory), or in a non-defended territory where only non-dominant males were present (undefended); data summarized in supplementary **Table 2**. Consistent with treatment-level measurements, there were significantly more offspring born within-territory than in extra-territory or undefended conditions (GLMM: $n = 36$; $F = 10.4$, $P = 0.0005$). Promiscuous line males had higher overall reproductive success than monogamous line males across all three territory types (GLMM: $n = 36$; $F = 8.13$, $P = 0.0083$) (**Figure 4c**). There was no territory type *x* paternal treatment interaction (GLMM: $n = 36$; $F = 0.3$, $P = 0.74$), and post-hoc Tukey's HSD tests showed no significant difference within any of the three conditions, indicating that greater reproductive success in promiscuous line males was driven by a moderate advantage in all three conditions.

Survivorship. There were no overall treatment effects on survivorship within males ($\chi^2 = 2.32$, $P = 0.13$) or females ($\chi^2 = 0.15$, $P = 0.70$). However, socially dominant males and females had greater survivorship relative to subordinates (males $\chi^2 = 26.7$, $P = <0.0001$; females $\chi^2 = 15.1$, $P = 0.0001$) (**Figure 5a, b**). A comparison of treatment effects of mortality among socially dominant males revealed significantly greater mortality in promiscuous line males relative to monogamous line males (**Figure 5c**; $\chi^2 = 5.11$, $P = 0.024$). There were no treatment effects on survivorship among socially dominant females ($\chi^2 = 0.26$, $P = 0.61$).

Mate choice (F_3)

The number of scent marks made by promiscuous line males (182.1 ± 40.4) was greater than that of monogamous line males (99.3 ± 29.3), and promiscuous line males also covered a greater area with scent marks (401.6mm^2 ± 91.4) than monogamous line males (255.8mm^2 ± 53.8), though the differences were not significant (GLM: number of

marks, $F = 2.11$, $P = 0.16$; total area covered, $F = 1.89$, $P = 0.19$). Scent mark number or area covered did not influence the time of female investigation (GLM: number $F = 0.054$, $P = 0.82$; area $F = 0.047$, $P = 0.83$).

Females spent a greater proportion of time in cages scent-marked by promiscuous line males than monogamous line males (**Figure 6a**; $t(10) = -3.33$, $P = 0.010$). The same females also had a greater portion of offspring sired by promiscuous line males (**Figure 6b**; $t(10) = -2.51$, $P = 0.036$). As is typical of mouse mate choice experiments, we found frequent mating bouts (50.66 ± 12.28 per trial) during estrous [48], but due to a slow recording rate we were not able to distinguish intromissions from ejaculations. There were no significant effects of male breeding treatment on the frequency of the observed mating bouts ($t(10) = -0.16$, $P = 0.88$)). Five out of the nine litters conceived were pure litters of single parentage, all of which had promiscuous line paternity (binomial sign test: $P = 0.002$). In the three litters of mixed parentage, there were no treatment effects on male reproductive success ($t(4) = 0.46$, $P = 0.68$).

Weight (F_3)

The effect of breeding treatment on weight in male and female offspring is shown in **Figure 7**, and results from GLM analyses are summarized in **Table 3**. There were significant effects of treatment, age, and their interaction. Promiscuous line males and females were on average lighter than monogamous line animals from 30 to 60 days after birth. However, post-hoc Tukey's HSD tests showed that promiscuous line males and females were significantly *heavier* at 10 days of age. Post-hoc comparisons at other time points were not significant.

Discussion

462 Following just two generations of reintroducing a wild-derived colony of
463 laboratory mice to the process of social selection, we identified substantial, sex-specific
464 lifetime fitness effects. The primary finding of this research is that, during direct
465 competition among second-generation animals, promiscuous line males had greater
466 mating success, but not greater viability, than monogamous line males; promiscuous line
467 females had fewer litters than monogamous line females, and a significant treatment by
468 sex interaction effect on reproductive success suggests a mild fitness load. Second, a
469 mate choice experiment among third-generation animals showed that, when given a
470 choice, females had odor and mating preferences for promiscuous line males over
471 monogamous line males. Third, promiscuous line mice were significantly heavier than
472 monogamous line mice 10 days after birth, but became lighter than monogamous line
473 mice after weaning. Finally, although we did not experimentally test for genetic or
474 nongenetic inheritance, transgenerational effects might have contributed to these
475 results. We discuss each of these differences in turn.

476 During competition promiscuous line males had greater mating success (**Figure**
477 **2a**), but also reduced survivorship (**Figure 5c**), relative to monogamous line males,
478 suggesting a functional trade-off between the expression of sexual traits and viability.
479 Surprisingly, the promiscuous line male advantage could not be explained by greater
480 success in acquiring social territories, nor could it be explained by sperm competition,
481 because promiscuous line males had no advantage in mixed litters (**Figure 4a**). Rather,
482 analysis of treatment-level reproductive success showed that promiscuous line males
483 had nearly exclusive (95%) paternity within territories defended by promiscuous line
484 males, while monogamous line males had only 68% of the offspring born within
485 territories defended by monogamous line males. Promiscuous line males also had
486 higher paternity in undefended territories (**Figures 3 and 4c**). Parentage analysis
487 confirmed that socially dominant promiscuous line males produced more “within-territory”

488 offspring than dominant monogamous line males, and further revealed that they had
489 greater success in mating outside their own territory (i.e. “extra-territory” **Figure 4c**).
490 Finally, promiscuous line males sired a greater number of litters, but not larger litters
491 (**Table 1**).

492 In wild mouse societies, mate choice is largely driven by estrus females, who
493 move between the territories of socially dominant males to sample prospective mates,
494 and there is little coercion by males [31, 33]. Together, our results suggest that the
495 promiscuous line male advantage was largely driven by female mating preference for an
496 attractive, “live fast die young” male phenotype. In support of this, females that settled
497 within territories defended by promiscuous line males were less likely to engage in extra-
498 pair matings than females settled in monogamous line male territories or in undefended
499 territories. We then confirmed a role of female preference with a mate choice experiment
500 on third-generation animals: when given a choice, females had both odor and mating
501 preferences for promiscuous line males (**Figure 6**).

502 Female fitness was relatively unaffected by breeding treatment during direct
503 competition; there were no overall effects on reproductive success, survivorship or social
504 dominance. Intriguingly, we found that promiscuous line females had significantly fewer
505 litters than monogamous line females (though they had slightly but nonsignificantly more
506 pups per litter). Consistently, our analysis demonstrated a significant treatment by sex
507 interaction effect, where lower reproductive success in promiscuous line females was
508 statistically correlated to higher reproductive success in promiscuous line males (**Figure**
509 **2c**). These sex specific results are consistent with the sexual conflict model, and support
510 results from experiments in flour beetles and amphipods showing social selection
511 pressures tend to enhance fitness in sons and deplete it in daughters [14, 18]. However,
512 our results contrast with studies on *D. melanogaster*, which often show strong negative
513 effects of social competition [15-17].

514 “Live fast die young” and "sexy son" male phenotypes are a classic signature of
515 sexual selection [49]. Two evolutionary genetic models of animal behavior could explain
516 the male phenotype we observed. First, the sexy sons hypothesis predicts that a male
517 who delivers no direct benefits (e.g., quality nesting sites) to the female can nevertheless
518 be favored if his sons are sexually attractive. Alternatively, Zahavi’s handicap principal
519 [24], predicts that sexually selected traits are honest indicators of health and vigor
520 because they handicap performance and survival. Here, sexually selected traits provide
521 a means for females to identify “good-genes” signals of quality in their mates because
522 expression of the trait is negatively correlated with performance. Results from our
523 experiment are perhaps more consistent with the sexy sons model, because
524 promiscuous line males had an advantage in mating success and in attracting females,
525 but were not of higher quality than monogamous line males. Furthermore, females of the
526 promiscuous line were not of higher quality than females from the monogamous line.
527 Importantly, these hypotheses are controversial because mathematical models [50] and
528 experiments [15] suggest indirect effects via offspring fitness have trivial evolutionary
529 effects relative to direct costs and benefits of female mating; our results add to growing
530 evidence that indirect fitness effects can be substantial [51, 52].
531 Although single-generation effects of sexual selection are often interpreted in
532 light of genetic evolution [e.g., 13], there is growing appreciation that the parental
533 environment *per se* can significantly impact offspring phenotype and fitness independent
534 of genetic inheritance, and that parental effects can ultimately influence the evolutionary
535 response to sexual selection [37]. This is especially true for the social environment [53],
536 and transgenerational effects have recently been reported in breeding experiments
537 similar to ours [54]. Maternal effects on offspring fitness can arise by changes in the
538 uterine hormonal milieu or nutritional investment in offspring, and effects on offspring
539 weight are particularly common [37]. We found evidence for such effects in our weight

analysis of F_3 mice. Promiscuous line males and females were significantly heavier than monogamous line offspring before weaning, but were lighter after weaning (**Figure 7**). Because birth weight usually predicts adult weight in mammals, this shift was unexpected. During our study pregnant promiscuous line females were transferred from seminatural enclosures to solitary cages to give birth, and would be expected to have different stress hormone profiles than monogamous line females [55]. These differences could induce differential investment in offspring. Consistently, maternal stress late in pregnancy has previously been associated with elevated birth weight, and this weight difference diminished by postnatal week ten [56].

We are not able to determine if the advantage enjoyed by promiscuous line males is due to genetic or transgenerational inheritance. Genetic selection could have occurred in the promiscuous line if particular alleles were strongly favored during social competition and sexual selection. Although MHC mating preferences have been observed in seminatural enclosures similar to ours [39], we eliminated any such potential effects by equalizing the frequency of MHC haplotypes between the two treatments. Also, although social dominance has been found to have a high narrow-sense heritability [57], we observed no selection on social dominance ability in our experiment. Finally, from our parentage analysis we found no significant differences in microsatellite heterozygosity between promiscuous line and monogamous line animals (data not shown). Nevertheless, other unidentified loci could have been under strong selection in this experiment. A non-mutually exclusive hypothesis is that transgenerational inheritance via maternal or paternal effects [58] could have increased mating success in promiscuous line males. We are currently investigating the role of transgenerational inheritance in this system.

Conclusion

565 Components of sexual selection such as female mate choice or male-male
566 competition can have dramatic effects on offspring development and fitness in the
567 laboratory, but less is known about the total effects of sexual selection in nature.
568 Although invertebrate studies that experimentally eliminate sexual selection using
569 enforced monogamy show that broad-sense sexual selection has strong and deleterious
570 effects on offspring fitness, these studies lack a critical feature of normal breeding
571 systems: competition for mating resources. Social selection is an extended form of
572 sexual selection that incorporates competitive social interactions and predicts complex
573 evolutionary dynamics [10, 11].
574 We report here new results on the effects of social selection on vertebrate
575 offspring fitness, and show that that sons conceived during social competition have a
576 fitness advantage by having greater reproductive success and attractiveness to females.
577 This latter effect involves chemical communication and, likely, the expression of
578 pheromones. We also identify fitness tradeoffs to male survivorship and female
579 fecundity. Although we did not identify the mechanism of inheritance, the emergence of
580 an adaptive phenotype within two generations of social selection is suggestive of
581 transgenerational inheritance.
582

583 Competing interests

584 The authors declare that they have no competing interests.
585

586 Authors' contributions

587 ACN and WKP conceived of the study. ACN carried out the experiments and
588 drafted the manuscript. KEC and SH participated in the direct competition and mate
589 choice experiments. WKP participated in coordination of the study. All authors read and
590 approved the final manuscript.

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Figure Legends

Figure 1. Breeding design. Monogamous boxes represent offspring conceived in random mate-assignment cages and promiscuous boxes represent offspring conceived in promiscuously breeding enclosures. See methods for details.

Figure 2. Reproductive success. A and B show Cumulative lifetime reproductive success; number of offspring over 35 weeks of competition for promiscuous line (open circles) and monogamous line (filled squares) males (A) and females (B). Male reproductive success values are calculated from number of sons only. C shows interaction of sex-specific effects of breeding treatment. Promiscuous line, circles; monogamous line, squares. Data are reproductive success means and standard errors per pup sweep cycle; male reproductive success values (determined by number of male offspring) were multiplied by two. The interaction term is significant ($P = 0.027$).

Figure 3. Effects of breeding treatment on social dominance and reproductive success. Promiscuous line males (open bars) had significantly more offspring than monogamous line males (closed bars) due to greater relative representation within territories defended by either promiscuous (P) line or monogamous (M) line males, and in undefended territories. Means and standard errors are calculated from 10 enclosures.

Figure 4. Mean number of offspring by litter type and territory type using parentage analysis. There were no effects of paternal breeding treatment on the size of mixed (A) or pure (B) litters. Individual promiscuous (P) line males (circles) had more offspring within their own defended territories (within-territory), inside the territories of neighboring dominant males (extra-territory), and in undefended territories than monogamous (M) line males (squares) (C). Means and standard errors are from two enclosures.

773 **Figure 5.** Effects of social dominance and breeding treatment on survivorship. Socially
774 dominant (half-filled circles) individuals had greater survivorship than subordinates
775 (triangles) for both male (A) and female (B) founders. Promiscuous line males that were
776 socially dominant (circles) had lower survivorship than monogamous line males that
777 were socially dominant (squares) (C).
778
779 **Figure 6.** Female preference for promiscuous line males. Females spent significantly
780 more time in cages scent-marked by promiscuous line males (A), and had more
781 offspring sired by promiscuous line males (B), relative to monogamous line males.
782 Transformed proportions were used in the analysis; raw proportions are shown for visual
783 purposes only.
784
785 **Figure 7.** Effect of breeding treatment on body mass before and after weaning.
786 Promiscuous line animals (circles) were heavier before weaning, but lighter after
787 weaning, than monogamous line animals (squares). Means and standard errors are from
788 an average of 10 caged mice each for females (A) and males (B).
789

Table 1. Male and female reproductive success. GLMM analysis of components of reproductive success with enclosure as a random effect and all other variables as fixed effects. *n* = 100.

		males				females			
Response Variable	**Source**	**DF**	**DFden**	***F***	***P***	**DF**	**DFden**	***F***	***P***
Number of offspring									
	Sweep	4	84.5	0.94	0.45	4	82.7	0.75	0.56
	Breeding treatment	1	84.4	4.38	**0.039**	1	82.1	0.70	0.41
	Breeding treatment *x* number of surviving males	1	41.8	4.11	**0.049**				
Number of litters									
	Sweep	4	83.1	1.02	0.40	4	85	0.55	0.70
	Breeding treatment	1	82.6	5.82	**0.018**	1	85	6.59	**0.012**
Litter size									
	Sweep	4	72.8	1.22	0.31	4		83.8	0.27
	Breeding treatment	1	75.8	0.25	0.62	1		83.3	0.28

Table 2. Results from parentage analysis. Shown are the effects of paternal breeding treatment on mixed (multiple paternity) or pure (single paternity) litter size and the effect of social dominance on number of offspring. GLMM analysis of reproductive success with population as a random effect and all else as fixed effects.

Response variable	**Source**	**DF**	**DFden**	***F***	***P***
Litter size					
	Sweep	4	92.4	0.64	0.64
	Maternal treatment	1	93	0.63	0.43
	Litter type (mixed or pure)	1	92	10.3	**0.0019**
	Maternal treatment x litter type	1	92.0	0.93	0.34
Mixed litter size					
	Sweep	4	12.7	0.36	0.83
	Maternal treatment	1	51.5	0.33	0.57
	Paternal treatment	1	51	0.87	0.35

	Maternal x paternal treatment	1	51	1.61	0.21
Pure litter size					
	Sweep	4	67.8	0.79	0.54
	Maternal treatment	1	70.1	0.16	0.69
	Paternal treatment	1	65.0	1.34	0.25
	Maternal x paternal treatment	1	54.3	0.95	0.34
Number of offspring by territory type					
	PIT-tag reader session	2	27	0.76	0.48
	Territory type*	2	27	10.4	**0.0005**
	Paternal treatment	1	27	8.13	**0.0083**
	Territory type x paternal treatment	2	27	0.30	0.74

*Territory type: Within-territory, extra-territory or undefended.

Table 3. Effects of breeding treatment on weight. GLM analysis with mass (g) as a dependent variable, and breeding treatment and age as fixed factors.

	females			**males**		
Source	SE	t	*P*	SE	t	*P*
Treatment	0.17	1.95	0.053	0.32	1.94	**0.054**
Age	0.010	10.0	**<0.0001**	0.0072	10.8	**0.018**
Treatment x age	0.011	2.36	**0.020**	0.0072	2.40	**<0.0001**